REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application.

The claims have been amended to correct the dependency thereof.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: VERSION WITH MARKINGS TO SHOW CHANGES MADE

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

A paragraph has been added before the paragraph beginning on page 1, line 1.

The paragraph beginning on page 13, line 13 has been amended as follows:

--The enzymatic reaction is conducted at pH 6.0 to [7.0] 11, preferably pH 7.0 to 10, and at temperatures of 4°C to 45°C, preferably 20°C to 40°C. Maltose can be used as a substrate in a concentration of less than 50%. The trehalose synthase enzyme can be used in a pure form or in crushed cells.--

The paragraph beginning on page 16, line 19, has been amended as follows:

--The pure chromosomal DNAs isolated from *Pseudomonas stutzeri* were partially digested with restriction enzyme *Sau*3AI at 37°C for 15 to 30 minutes. The restriction enzyme was inactivated with heat and agarose gel electrophoresis was carried out to obtain 3 to 10 kb DNA fragments. As shown in Figure 5, plasmid pUC18 was digested with *Bam*HI and was treated with calf intestinal phosphatase. The cleaved DNAs were mixed with 3 to 10 kb DNA fragments previously obtained and ligation with T4 DNA ligase was allowed at 15°C for 16 hours. The recombinants thus obtained were used for transofrmation. The transformation was

carried out by electroporation as follows. *E coli* NM522 was cultured on LB medium for 14 to 15 hours. The resulting culture was inoculated on 1L LB so that initial absorbency became 0.07 to 0.1 at 600 nm, and then cultivation was allowed until the absorbency reached 0.8. The cells were centrifuged and suspended in 1L of HEPES [N-(2-hydroxyethyl)piperazine-N-(2-ethanesulfonic acid)] buffer solution. The cells were again centrifuged and suspended in 500 ml of cold sterile deionized distilled water. The cells were again centrifuged and suspended in 20 ml of 10% glycerol solution. The cells were again centrifuged and suspended in 2 to 3 ml of 10% glycerol solution so that the cell concentration was adjusted to 2-4 x [1,010]10^{10}/ml. The cell suspension was rapidly frozen and stored at -70°C. The frozen cells could be used for about one month during which time their transformation frequency did not decrease. 40μL of frozen cell suspension was thawed in ice and the restored suspension was mixed with the ligated DNA solution. The mixture was put in a gene pulser cuvette with a diameter of 0.2 cm and the capacitance and strength of electric field was fixed at 25 uF and 12.5 kV/cm, respectively. After a single electric pulse was passed at resistance of 200 to 400 Ω, 1 ml of SOC medium was immediately added and cultured at 37°C for 1 hour. The culture was streaked on LB-ampicillin agar medium and cultivation was allowed for 24 hours to obtain at least fifty thousand colonies. These colonies were together cultured in LB broth for 2 hours. DNA was purely

isolated using an alkaline lysis and the genomic library was constructed therefrom.--

The table beginning on page 18, line 5, has been amended as follows:

--Table 3. Enzyme Titration

Microorganisms	[Non-enzymatic activities] Specific activity of enzyme (U*/mg of protein)	Culture Titer (U/ml of culture solution)
Pseudomonas stutzeri CJ38	0.1	0.023
E. coli ATCC35467/pUC18	0	0
E. coli ATCC35467/pCJ104	0.26	0.175

*U-μmol trehalose/minutes--

The paragraph beginning on page 18, line 22, has been amended as follows:

--The plasmid pCJ104 was subjected to single, double, and triple-digest procedures using about twenty restriction enzymes, such as *Aat*II, *Bam*HI, *Bgl*II, *Sma*I, *Eco*RI, *Eco*RV, *Kpn*I, *Nco*I, *Nde*I, *Pst*I, *Sac*I, *Sac*II, *Sal*I, *Sph*I and *Xho*I. DNA fragments were analyzed by electrophoresis through agarose gel and compared to construct the restriction map.--

The heading beginning on page 18, line 28, has been amended as follows:

--**Example [3]8**--

The table beginning on page 20, line 17, has been amended as follows:

--Table 5

Microorganisms	[Non-enzymatic activities] Specific activity of enzyme (U/mg of protein)	Culture Titer of 5 L Fermenter (U/ml of culture)
E coli ATCC35467/pCJ121	0.43	-
E. coli ATCC35467/pCJ122	4.95	30
E. coli ATCC35467/pCJ123	0	-

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In the Claims:

The claims have been amended as follows:

3. (Amended) A recombinant plasmid containing the trehalose synthase gene of claim [1] 2.

4. (Amended) The recombinant plasmid according to claim [1] 3 which is recombinant plasmid pCJ122.

5. (Amended) A transformed E. coli with the recombinant plasmid of claim [1] 3.